



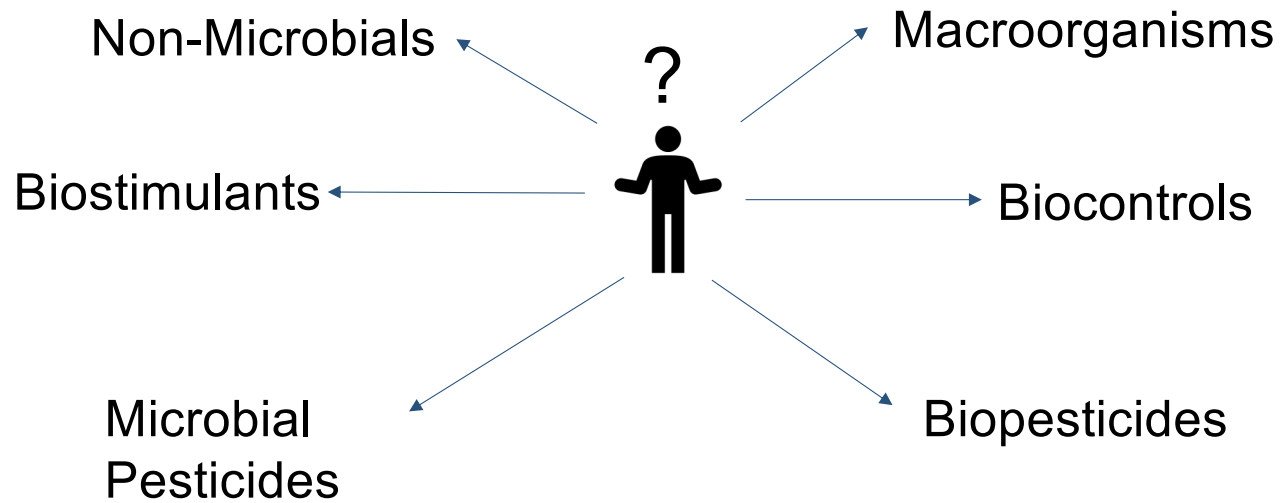
A CRO Perspective and Insights into Biological Product Registration and Testing with an Emphasis on Relevant Environmental Exposure

Henry “Hank” Krueger
Eurofins Agrosience Services

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BIOLOGICAL PRODUCTS ?



BIOLOGICAL PRODUCTS

Source: DunhamTrimmer®, LLC

BIOSTIMULANTS ¹				BIOCONTROLS						
MICROBIALS		NON-MICROBIAL		BIOPESTICIDES ³		MACROORGANISMS ⁶				
NUTRIENT USE EFFICIENCY (NUE) (BIOFERTILIZERS) ²	PLANT GROWTH PROMOTION (PGP)	PLANT & SEAWEED EXTRACTS	AMINO ACIDS	BIOCHEMICALS ⁴	MICROBIALS ⁵		INSECTS	MITES	NEMATODES	
		ORGANIC ACIDS	INORGANIC COMPOUNDS	PLANT EXTRACTS	BACTERIA	FUNGI	<p>5 Microbials refer to products based on bacteria, fungi, viruses, and protozoans. Microbials comprise the largest market of biopesticides.</p> <ul style="list-style-type: none"> • Bacteria, followed by fungi, make up the largest groups commercially (>90%). • Biggest challenges relate to product formulation with regard to shelf-life, stability, and performance enhancement. 			
<p>1 Biostimulants are products which elicit one or more of the following effects: 1) mitigate abiotic stress; 2) enhance crop quality; 3) improve nutrient assimilation. Their functions are typically classified as NUE (Nutrient Use Efficiency) or PGP (Plant Growth Promotion).</p>		ORGANIC ACIDS	PGRs	PROTOZOA	VIRUS					
		SEMICHEMICALS	YEASTS	OTHERS						
<p>2 Biofertilizers are Microbials used to enhance plant nutrient uptake from soil (NUE).</p> <ul style="list-style-type: none"> • N-fixing bacteria make up the largest segment. • N-fixing bacteria for non leguminous crops make up the fastest growing segment. • Other NUE microbials include mobilizers and solubilizers or chelators of specific nutrients such as P, K, S, Zn, Fe. <p>PGP Microbials target other biostimulant properties beyond NUE.</p>		<p>Non-microbial biostimulants may target either NUE or other PGP effects.</p> <ul style="list-style-type: none"> • Amino Acids and Seaweed Extracts are the fastest growing segments. • Seaweed Extracts are a complex mixture of components including plant hormones, phenolic compounds, and other active substances. • Amino Acid products include peptide fractions. • Organic acids are mainly humic and fulvic acids used as soil amendments. 		<p>3 Biopesticides are derived from natural materials such as plants, bacteria and certain minerals. Biopesticides target specific pests and are inherently less toxic than synthetic pesticides.</p>		<p>4 Biochemicals include Plant Extracts (largest by sales volume), Organic Acids, PGRs (plant hormones e.g. cytokinins, auxins, etc), and Semiochemicals (allelochemicals and pheromones).</p>		<p>6 Macroorganisms include insects, mites, and nematodes. Insects & mites are the largest groups.</p> <ul style="list-style-type: none"> • Unique in that the live organism is used in the form of eggs, larvae, pupae, or adults. • The most important challenge in this category is logistics — shipping live organisms that require special care to survive. • Normally not classified as Biopesticides but rather Biocontrols. 		



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 International Bio Intelligence

- Biostimulants – do not carry pesticidal claims, so are not regulated under FIFRA, but are regulated by states
- Biocontrol Products
 - Macro-organisms importation, transport and release are regulated by USDA
 - APHIS regulation of biological control organisms is based on Plant Protection Act of 2000 U.S.C. 7701 et seq.) APHIS has broad authority to regulate plant pests, including “indirect plant pests”
 - Types of Biological Control Agents. Natural enemies of insects and mites generally fall into four different types, or guilds, based on how they utilize their prey or hosts: predators, parasites, parasitoids, and pathogens INSECTS, MITES AND NEMATODES.
 - Biopesticides carry pesticidal claims and are regulated under FIFRA. Outdoor uses require EPA's ecotoxicity test battery.

BIOCONTROLS

BIOPESTICIDES ³				MACROORGANISMS ⁶		
BIOCHEMICALS ⁴		MICROBIALS ⁵		INSECTS	MITES	NEMATODES
PLANT EXTRACTS		BACTERIA	FUNGI			
ORGANIC ACIDS	PGRs	PROTOZOA	VIRUS			
SEMIOCHEMICALS		YEASTS	OTHERS			

5 Microbials refer to products based on bacteria, fungi, viruses, and protozoans. Microbials comprise the largest market of biopesticides.

- Bacteria, followed by fungi, make up the largest groups commercially (>90%).
- Biggest challenges relate to product formulation with regard to shelf-life, stability, and performance enhancement.




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International Bio Intelligence

EPA Evaluates Test Materials Using A Deterministic Approach

RISK QUOTIENT = EXPOSURE / TOXICITY

Calculation of risk quotients are based upon ecological effects data, pesticide use data, fate and transport data, and estimates of exposure to the pesticide. In this method, the estimated environmental concentration (EEC) is compared to an effect level, such as an LC₅₀ (the concentration of a pesticide where 50% of the organisms die.) This ratio is a simple, screening-level estimate that identifies high- or low-risk situations.

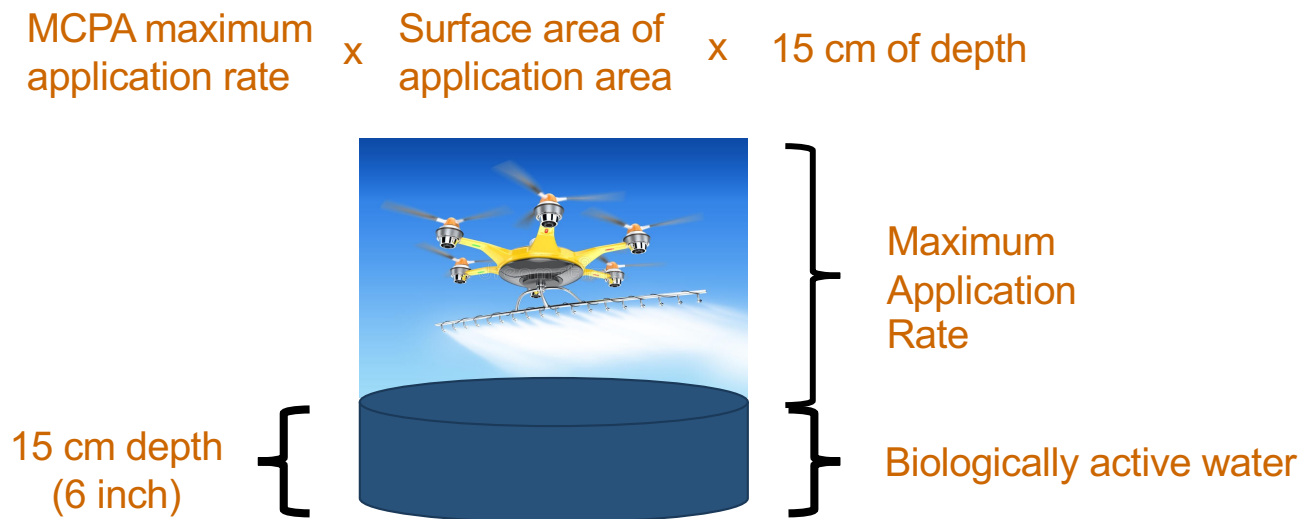
Contract Lab Perspective - - MCPA Testing in General and ESA in Particular

- 
- 1) Cannot Test Endangered Species and need to rely on guideline test species
 - 2) Need to set test concentrations and evaluate risk based on realistic exposures
 - 3) Need to develop exposure models and use pre-test trials to design better tests to evaluate exposure

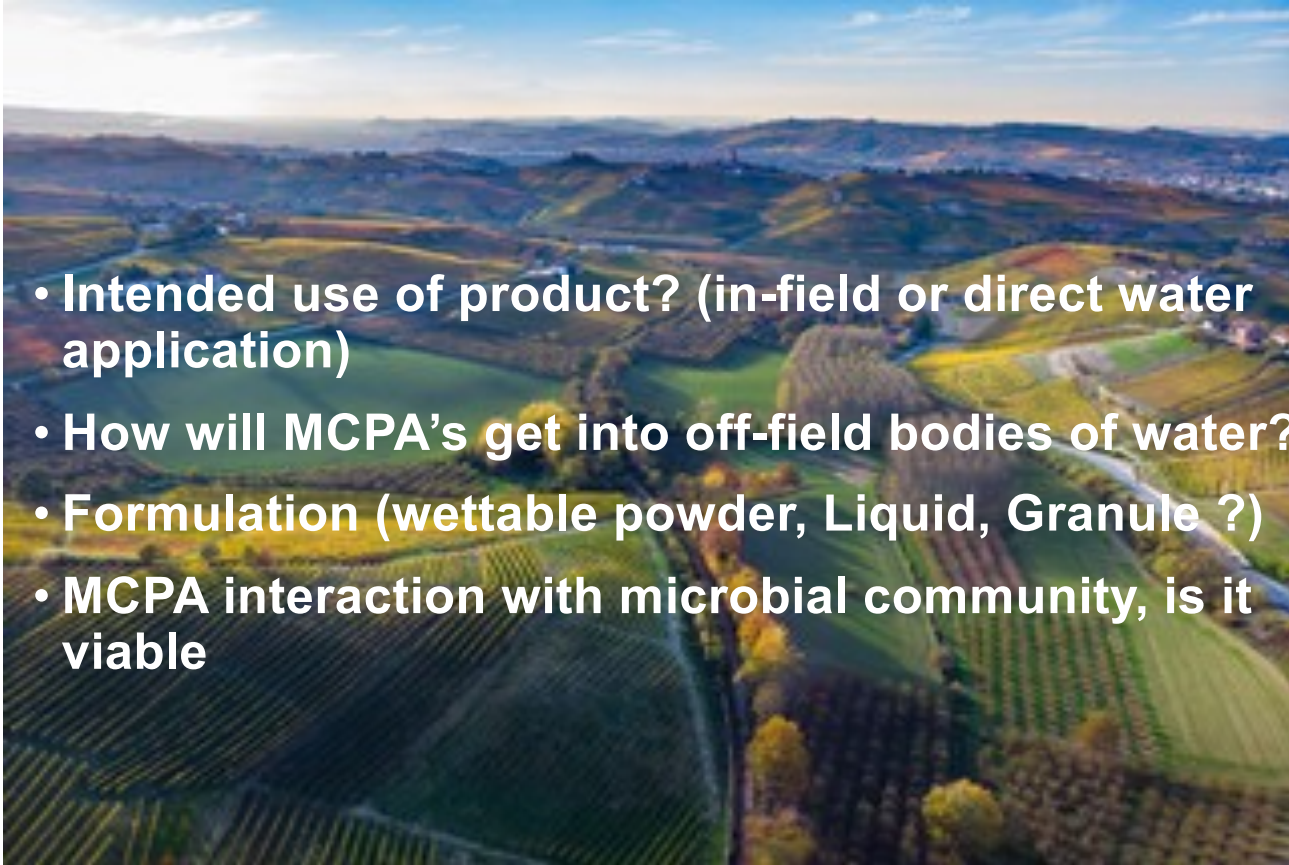
- **Regulatory agencies use the maximum hazard concentration (MHC) for setting the high concentration for Aquatic organisms.**
- **Water**
 - **MHC = 10^6 units/mL or,**
 - 1000 times the maximum calculated pesticide concentration in water immediately following a direct application to a 6-in layer of water
- **Food**
 - MHC = 100 times the expected microorganism concentration in the aquatic environment
 - 100 times the maximum calculated pesticide concentration in water immediately following a direct application to a 6-in layer of water

Do these MHC values make sense?

- These values are old and go back to the original OPPTS guidelines of the mid-90s
- We have learned a lot since then and have greatly changed application methods to reduce environmental exposure



Factors to Consider in an Exposure Assessment

- 
- Intended use of product? (in-field or direct water application)
 - How will MCPA's get into off-field bodies of water?
 - Formulation (wetable powder, Liquid, Granule ?)
 - MCPA interaction with microbial community, is it viable

Greatest exposure is direct application to water



(e.g. mosquito control)

Next highest exposure is broadcast applications



Aerial applications vis plane or drone



Foliar boom spray applications

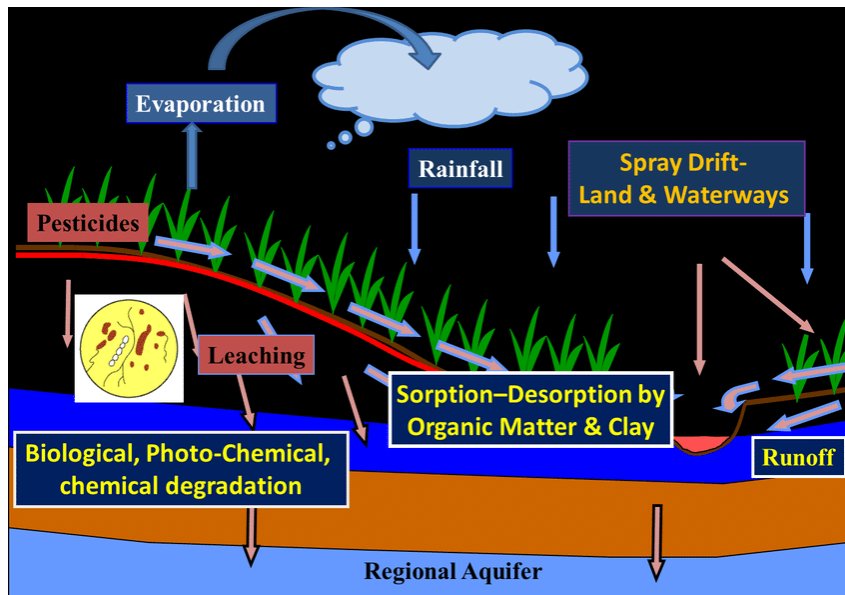
Lower Exposure for In-Furrow Application



Lowest Exposure is Seed Treatment



Routes of Exposure are well defined for conventional chemicals

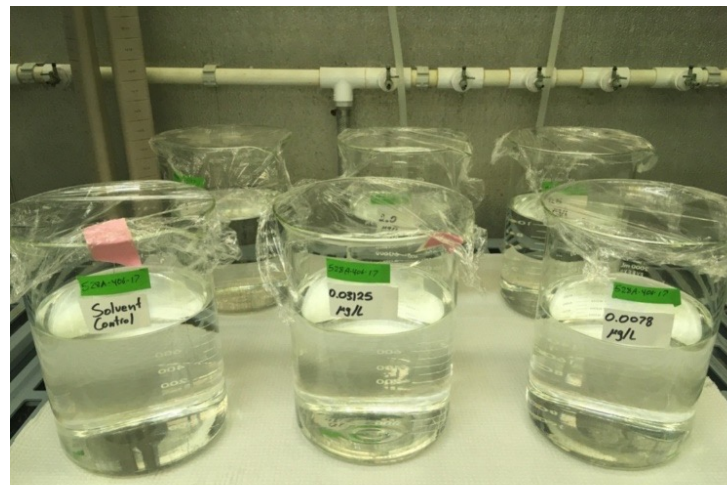


Computer models and e-fate data are used to determine estimated environmental concentration (EEC)

Need tools to estimate environmental concentrations for MCPA's under different application scenarios.

GLP Inputs for Exposure Models With Conventional Chemicals

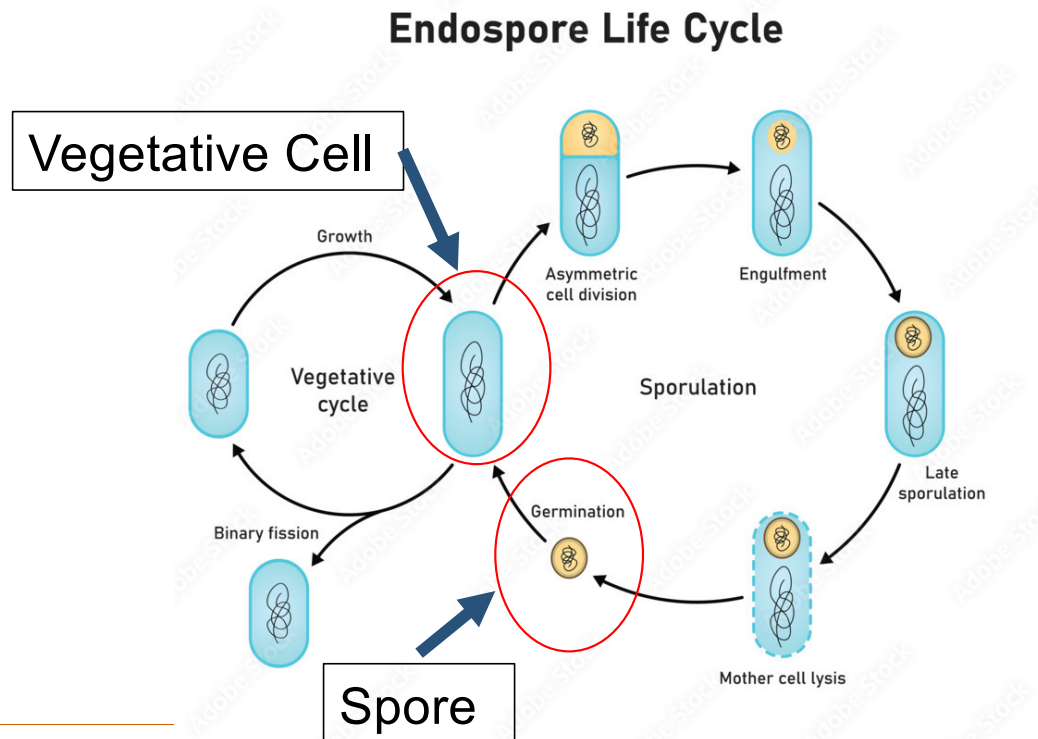
- Water solubility
- Degradation
 - Photolysis
 - Hydrolysis
- Volatility- Henry's Law
- Kow – octanol-water partition
- Koc – soil organic carbon – water partition coefficient



These studies do not exist or apply for MCPA's.

Need to develop tests to help improve exposure assessments and help design tests

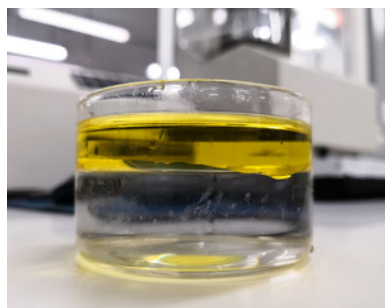
How will the microbe react in water ?



Pre-Test studies for MCPA's that can help in the design of better tests

Determine if MCPA floats, sinks, or is suspended in water

- Sinks – sediment exposure more likely
- Floats – exposure to the sun's UV-radiation (may affect viability)
- Suspends – water exposure



Viability – in test system and growth

Settling time

Stability – in test system

Homogeneity

Need for aeration/effect of aeration

Turbidity- color and visibility

Need for filtration

Assess Controls

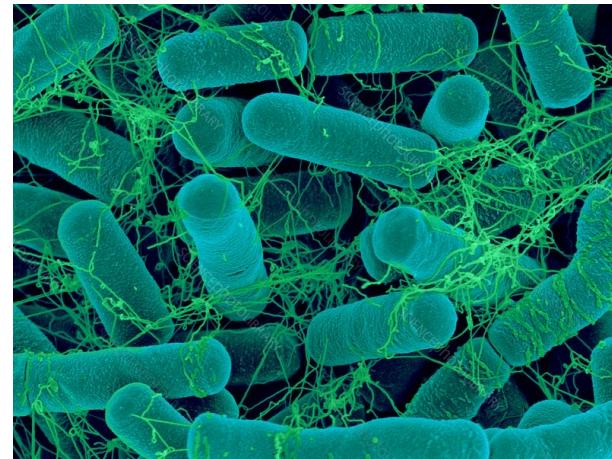
Time interval before test organisms are added to test vessels

Collect and analyze samples during trials



Conclusions

- Routes of exposure and estimated environmental concentrations need to be used to both determine how high to test and evaluate risk.
- There is a need for exposure models.
- Pretest experiments should be run to evaluate exposure to help design tests and determine if testing is warranted



<https://www.sciencephoto.com/media/798570/view/bacillus-thuringiensis-soil-bacterium-sem>



Microbial Testing Challenges and Endangered Species Assessments

Lisa Ortego, PhD, DABT

Environmental Toxicologist/Risk
Assessor

Bayer CropScience





US EPA Approach to Biopesticide Testing and Assessment

US has separate guidelines (Series 885) for biopesticide versus pesticide chemicals (Series 850) evaluates both ecotoxicity and pathogenicity (for microbials)

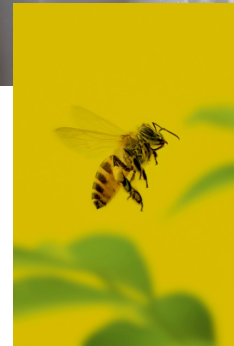
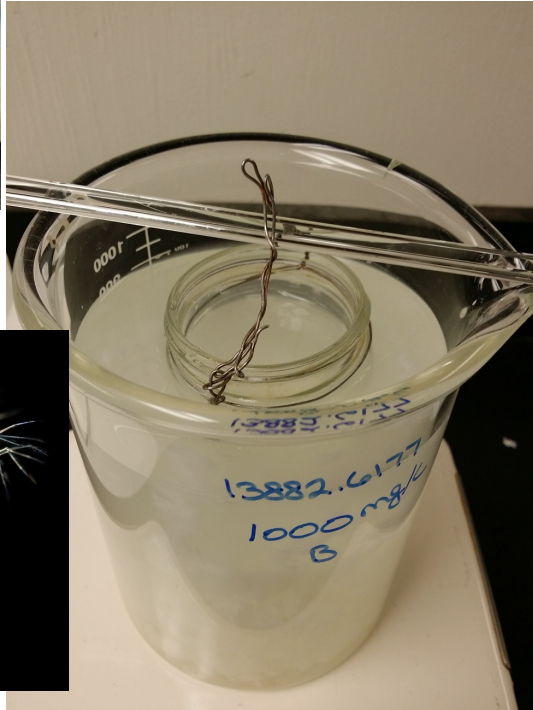
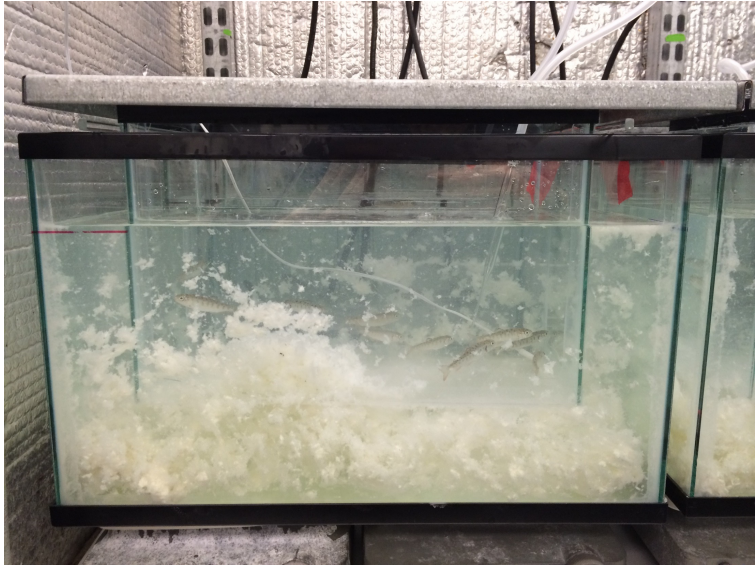
Standard environmental exposure models are not validated for microbials and are not used to estimate environmental exposure

In lieu of quantitative exposure estimates in the risk assessment, testing is required at high test concentrations (maximum hazard concentration or MHC); based on multiples of the use rate (100X for example)

// Much higher exposure than would be anticipated under labeled use conditions

Testing is designed to determine if pathogenic or toxic effects occur at MHC; if no effects, safety is presumed

However, there are challenges with testing microbials especially at such high levels.





Typical Tests and Their Challenges

Aquatic Testing

- // Distinguish between toxicity and pathogenicity
- // Non-specific effects due to particulate nature of test substance
- // Turbidity may interfere with observations

Algae Testing

- // Bacterial competition for nutrients
- // Turbidity may interfere with instrumentation

Bee Testing

- // Bee diet may be unpalatable
- // Distinguish between toxicity and pathogenicity
- // Test duration may be insufficient to explore possible pathogenicity

Non-specific effects can be confused with effects due to test substance exposure suggesting risks where none exist – will complicate endangered species assessments



Initiatives To Improve Testing and Assessment - OECD

OECD – via Expert Group Biopesticides (EGBP) part of the Working Party Pesticides

- // Expert group works to identify and fill gaps in testing and assessment – meets annually
- // Manual on Concepts and Available Guidance for Microbial Pesticides - OECD (<https://web-archive.oecd.org/temp/2023-04-21/652001-guidance-microbial-pesticides.htm>)
- // Sponsored conference in 2022 – Innovating Microbial Pesticide Testing
 - // High priority needs were identified by the Expert Group as an outcome of this conference
 - // Proceedings published by OECD ([https://one.oecd.org/document/ENV/CBC/MONO\(2023\)10/en/pdf](https://one.oecd.org/document/ENV/CBC/MONO(2023)10/en/pdf))
 - // Special issue of *Environmental Sciences Europe* includes publications from this conference
 - // <https://www.springeropen.com/collections/rampii>





OECD EGBP Workplan

Environmental Effects

Guidance document on best practices for ecological testing

Guidance document to determine when an *in vivo* test is needed (human and environmental safety)

Revise aquatic invertebrate guidance

Develop a list of secondary metabolites of concern

Develop a list of non-target insect species amenable to laboratory testing

Improved testing and accurately interpreting results are key components to addressing risk to endangered species

Health for all, Hunger for none



bayer.com

Thank you



Evaluating Biopesticides in California

Alex Magliano, Senior Environmental Scientist (Specialist)

CDPR, Ecotoxicology Program

April 2, 2025

Endangered Species and California

- ◆ California Environmental Quality Act (CEQA)
- ◆ California Endangered Species Act (CESA)
- ◆ DPR evaluation program is “CEQA Equivalent”



Photo by USFWS



Photo by Frank Schulenburg



Photo by John Cleckler, USFWS

Endangered Species and DPR



Photo by B. Peterson, USFWS

- ◆ Pesticide Regulation's Endangered Species Custom Realtime Internet Bulletin Engine (PRESCRIBE);
- ◆ Education and outreach
- ◆ Consulting and coordinating with federal and state regulatory agencies

How Does Ecotox Evaluate Biopesticides?

- ◆ Ecotoxicology Program assesses potential risks to non-target wildlife
- ◆ No specific endangered species assessment
- ◆ State-wide assessment considering the highest risk scenario.



Photo by Alex Magliano

Challenges Evaluating Biopesticides

Toxicity

- ◇ Relating lab concentrations to application rates
- ◇ Lethal and sublethal effects
- ◇ pathogenicity

Exposure

- ◇ Application rates
- ◇ Lack of environmental fate data
- ◇ Live organisms

Data Requirements



- ◇ Ecotoxicology evaluates Non-target organism toxicity tests
- ◇ Limited dataset
- ◇ Different guidelines for biopesticides

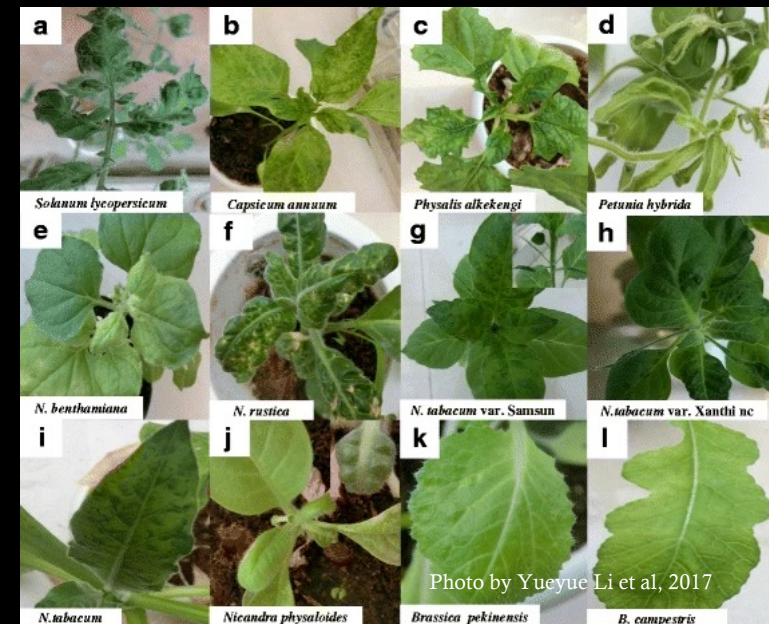
Waiving Required Data

- ◇ Claims must be supported by data
- ◇ How does it support the waiver request?
 - ◇ Ubiquitous in the environment
 - ◇ Genus/species
 - ◇ Optimal growth conditions
 - ◇ Host specificity
- ◇ Cite sources and be specific



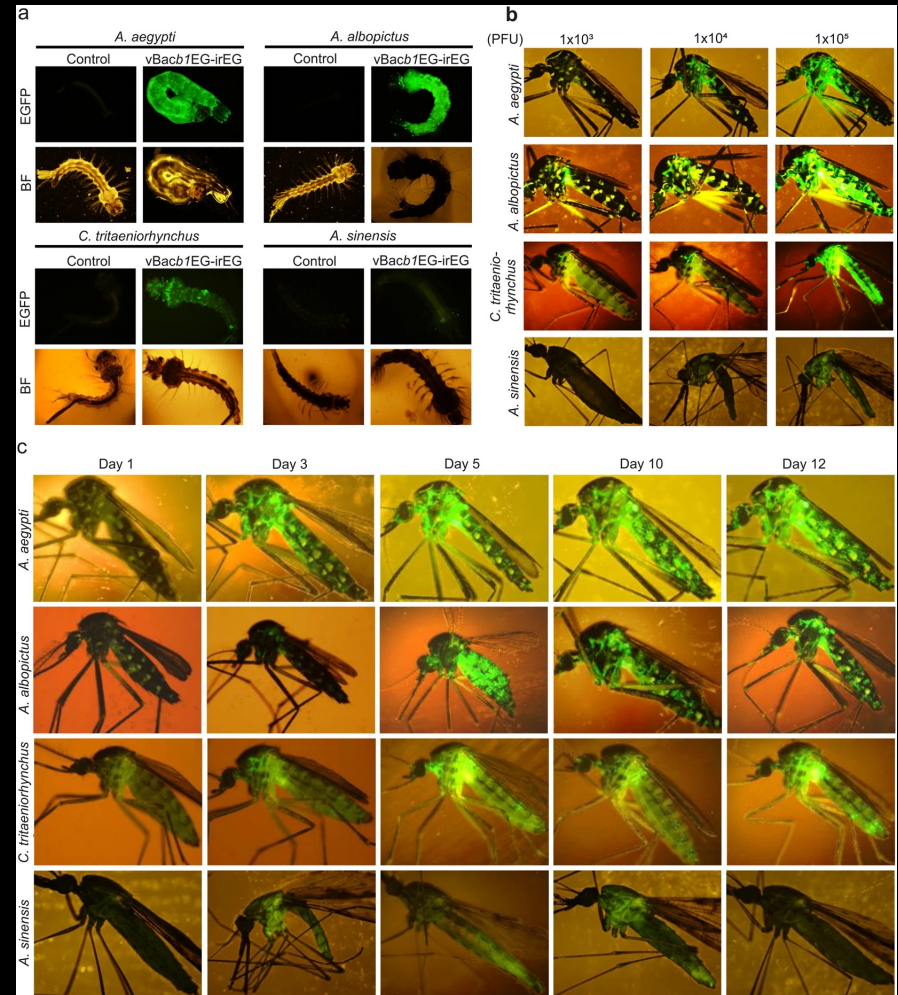
Examples

- ◇ Funnel web toxin
- ◇ Viral inoculation
- ◇ Capsaicin mountain lion repellent
- ◇ Case Study: Modified Mosquitos
 - ◇ Whole organism
 - ◇ Population-level control



Modified Mosquitos

- ◇ Product Characterization
 - ◇ Species, AI, mode of action, etc.
- ◇ Direct effects
 - ◇ Contact, ingestion, bites
- ◇ Indirect effects
 - ◇ Non-native,
 - ◇ no-ecosystem function,
 - ◇ prefers humans



Naik et al. *Sci Rep* (2018)



Thank You



FIFRA and the Endangered Species Act (ESA): What it means for Biologics

Annie Krueger

Compliance Services International



Endangered Species Act

Establishes protections for fish, wildlife, and plants that are listed as threatened or endangered by the U.S. Fish and Wildlife Service (FWS) and National Marine Fisheries Service (NMFS)

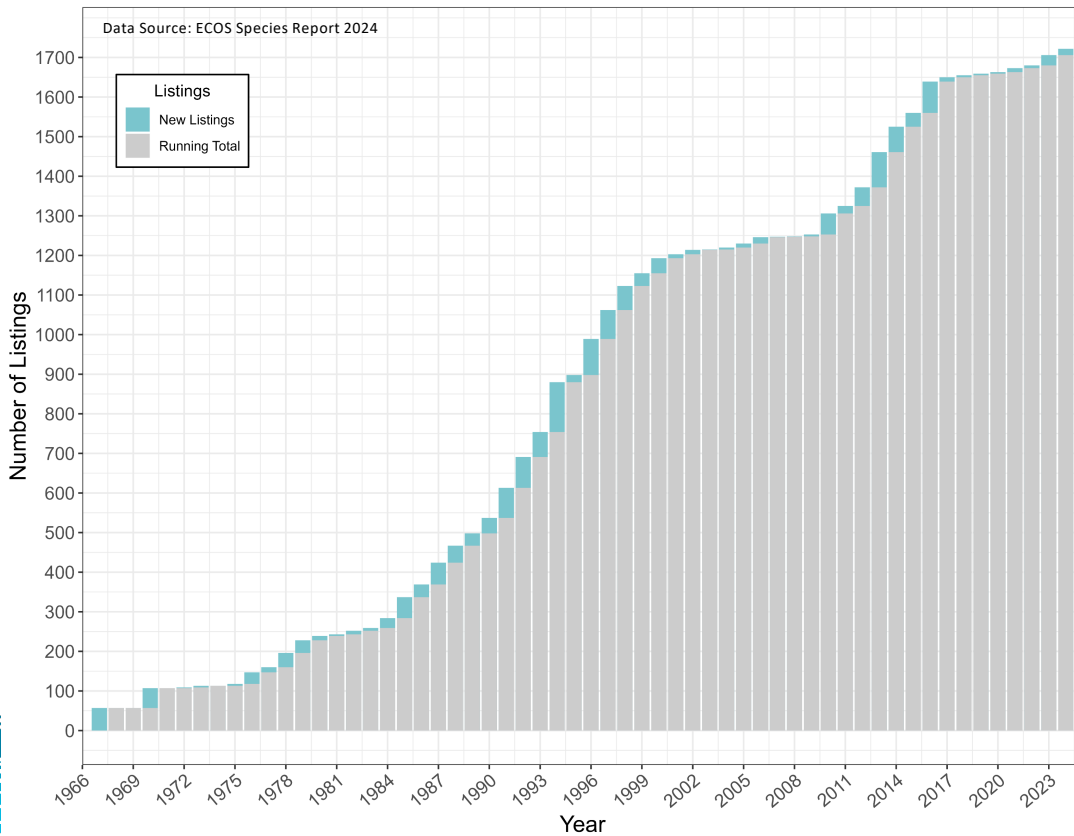


Photo Credit: USFWS ECOS Species Pages

Endangered Species Act

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Number of ESA Listed Species Over Time



Threatened & Endangered Species

Species Selected
 1,742

Critical Habitat Selected
 872

Additional Species Found in Current Map Extent

Amphibians

48

Arachnids

11

Birds

94

Clams

136

Conifers

5

Corals

1

Crustaceans

34

Ferns

37

Fish

174

Flowering Plants

895

Insects

101

Lichens

2

Mammals

94

Reptiles

56

Snails

54

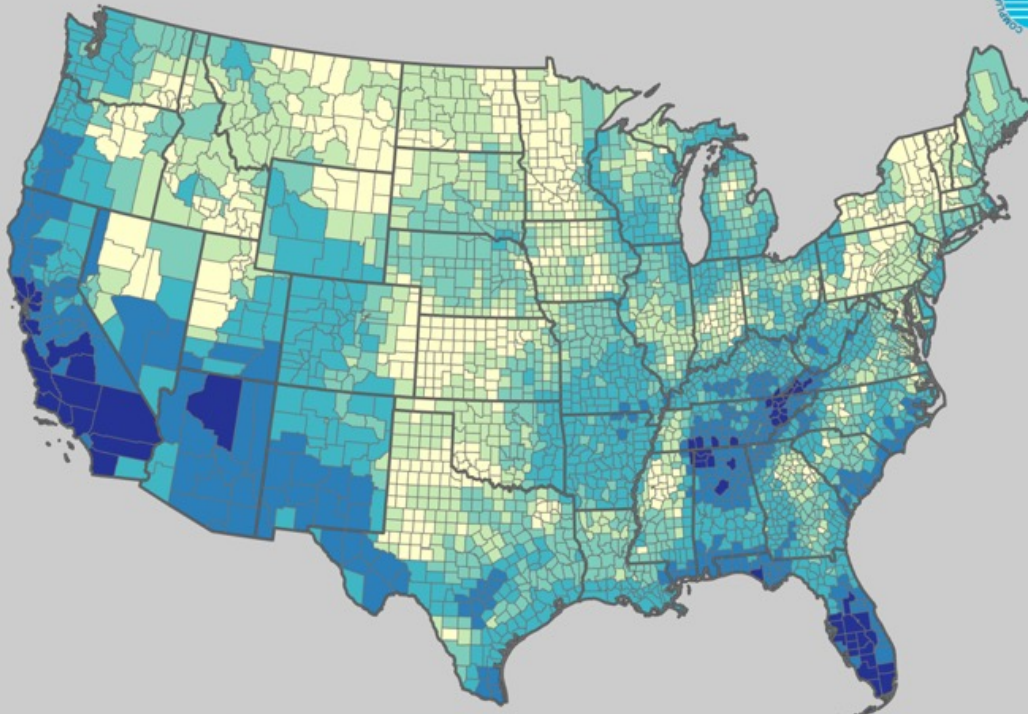
EPA OCSPP UDL Dashboard (<https://experience.arcgis.com/experience/f60e75af70b04a67955b32c1c083d1cd/>)



Endangered Species Act

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USFWS/NMFS SPECIES RANGE DENSITY



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Endangered Species Act

Endangered Species Act - Section 7

Requires Federal Agencies to consult with the Services (NMFS and FWS) on any “action” that may affect species listed as endangered or threatened

EPA

Must assess effects
to listed species for
any FIFRA pesticide
registration decision



Endangered Species Act

Endangered Species Act - Section 7

Requires Federal Agencies to consult with the Services (NMFS and FWS) on any “action” that may affect species listed as endangered or threatened

Any action EPA conducts must comply with Section 7, including the registration of biological products

“Will the registration have any effect on threatened and endangered species?”

No Effect

May Affect

EPA

Must assess effects to listed species for any FIFRA pesticide registration decision



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No Effect

No Toxicity
or
No Exposure

May Affect

Toxicity
and
Potential Exposure

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No Effect

No Toxicity
or
No Exposure

May Affect

Toxicity
and
Potential Exposure

>15 examples of biologics going through this process

Plant
Incorporated
Products

Microbials

Biochemical
Products



Endangered Species Act

Endangered Species Act - Section 7

“Will the registration have any effect on threatened and endangered species?”

EPA

Must assess effects to listed species for any FIFRA pesticide registration decision

No Effect

Toxicity

Exposure

Ledprona (dsRNA)
September 2023

“Ledprona dsRNA presents low or no toxicity to most nontarget organisms with the exception of beetles closely related to the target pest.”

“Specific habitat and life history indicates that there is negligible exposure to the listed beetles”

Therefore, **EPA is making a “No Effect” determination** under the Endangered Species Act (ESA) for all listed species and their designated critical habitats resulting from the uses of *Calantha* containing Ledprona.



Endangered Species Act

Endangered Species Act - Section 7

“Will the registration have any effect on threatened and endangered species?”

EPA

Must assess effects to listed species for any FIFRA pesticide registration decision

May Affect

Toxicity

Exposure

Beauveria bassiana
March 2023

“Since some adverse effects were observed in the nontarget insect studies, a no observed adverse effect concentration (NOAEC) cannot be established at or below the estimated environmental concentration (EEC) on the treated palm trees.”

EEC = the concentration of *Beauveria bassiana* strain 203 in the EP.

Therefore, the Agency made a “may affect, not likely to adversely affect” determination for listed insects at the treatment site.



Endangered Species Act

Endangered Species Act - Section 7

“Will the registration have any effect on threatened and endangered species?”

EPA

Must assess effects to listed species for any FIFRA pesticide registration decision

May Affect

Toxicity

Exposure

S18 – DQB Males
March 2023

“However, as DQB Males are intended to suppress *Cx. quinquefasciatus*, which is a primary vector of avian malaria, EPA concluded that there is the **potential for beneficial indirect effects to listed avian species** in the proposed treatment areas, as the reduction of *Cx. quinquefasciatus* could lead to a reduction in transmission of avian malaria.”

Because use of DQB Males could result in reduction of avian malaria, a disease which negatively impacts listed avian species, EPA made May Affect but Not Likely to Adversely Affect (NLAA) determinations for 29 listed avian species in the proposed action area due to the potential for beneficial effects



Endangered Species Act

Endangered Species Act - Section 7

“Will the registration have any effect on threatened and endangered species?”

EPA

Must assess effects to listed species for any FIFRA pesticide registration decision

May Affect

Toxicity

Exposure

Red Thyme Oil
August 2024

“Phytotoxicity was observed in the seedling emergence study conducted with an EP containing both Ais...
Based on an application rate of 7.0 lb EP/acre, risk quotients for terrestrial and semi-aquatic plants range from <0.1 to 8.1. The results specifically indicate that there is potential for risk to non-listed and listed dicot plant species from exposure to the proposed EP.”



Endangered Species Act

Endangered Species Act - Section 7

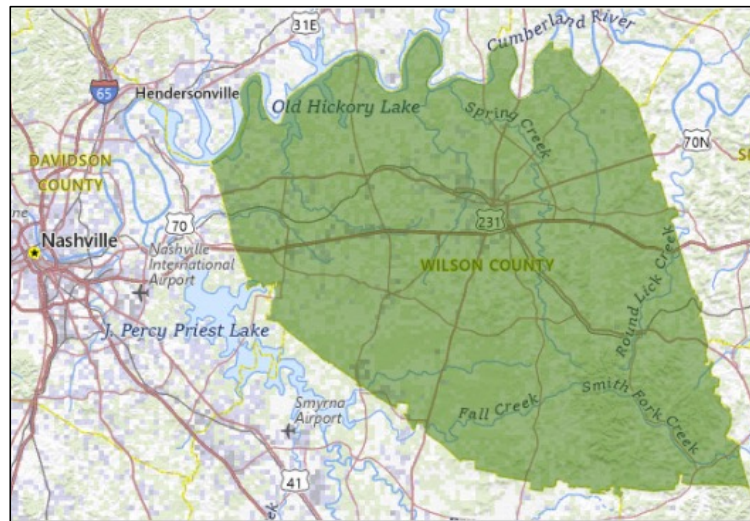
“Will the registration have any effect on threatened and endangered species?”

May Affect

Red Thyme Oil
August 2024

EPA

Must assess effects to listed species for any FIFRA pesticide registration decision



Additionally, the label includes use restrictions for Wilson County, Tennessee, to avoid on-field exposure for the listed Spring Creek bladderpod (*Lesquerella perforata*) that may be present on agricultural lands.

“Do not apply NSTKI-014 within Wilson County, Tennessee from September 15 to May 15.”



Endangered Species Act

Endangered Species Act - Section 7

“Will the registration have any effect on threatened and endangered species?”

May Affect

Red Thyme Oil
August 2024

EPA

Must assess effects to listed species for any FIFRA pesticide registration decision

Required Labeling Mitigations to Minimize Off-field Exposure:

- 1) The height of application is to be no more than 3 feet from the target vegetation in order to minimize drift.
- 2) The product must be applied with nozzles that dispense medium to coarse droplet sizes (Dv0.5 of 341 µm) to minimize drift distances.
- 3) **Applicators must maintain a 7-foot buffer strip between the point of direct application and the closest downwind edge of off-field habitats in order to minimize drift.**
- 4) For home and garden uses label and the turf and ornamental uses, the label has the following language: “apply directly to turf, ornamental plants, and fruit or vegetable plants via handheld sprayer only.”

“**Based on the label mitigations**, the Agency does not expect any on field or off-field risk to listed plants...Therefore, the EPA made NLAA determinations for the remaining 465 listed species and 128 designated critical habitats.”



Endangered Species Act

Endangered Species Act - Section 7

“Will the registration have any effect on threatened and endangered species?”

What can you do to prepare?



Endangered Species Act

Endangered Species Act - Section 7

“Will the registration have any effect on threatened and endangered species?”

What can you do to prepare?

No Effect

May Affect

Data on toxicity and exposure will form the basis of the assessment



Endangered Species Act

Endangered Species Act - Section 7

“Will the registration have any effect on threatened and endangered species?”

What can you do to prepare?

May Affect

Toxicity
and
Potential Exposure



Endangered Species Act

Endangered Species Act - Section 7

Requires Federal Agencies to consult with the Services (NMFS and FWS) on any “action” that may affect species listed as endangered or threatened



Thank You!

akrueger@complianceservices.com

EPA has been incorporating ESA into all their registration actions

EPA must apply FIFRA toxicity and exposure data to assess effects to ESA species

EPA has identified “May Affect” concerns for some biologics and required mitigations

